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Evaluation of wood treaed with copper-based preservatives for Cu loss during exposure to heat and copper-tolerant *Bacillus licheniformis*

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Abstract

Copper-based wood preservatives need to be effective against exposure to all types of microorganisms. Wood treated with six copper-based preservatives was exposed to 121°C and 20 psi pressure for 15 minutes under standard autoclave conditions and the copper-tolerant bacterium, *Bacillus licheniformis* CC01, for 10 d at 28°C and 150 rpm Sixteen to 37 percent of the copper was released from the wood during autoclaving, with copper citrate demonstrating the highest percent loss. Forty-four to 82 percent of the copper remaining in the samples following autoclaving was remove during exposure to the bacterium in liquid culture; copper naphthenate in oil and ACQ-D had losses of eighty percent or greater o the remaining copper. The bacterium removed as much or more total copper in 4 of 6 gas-sterilized samples (85-94%) than the cumulative effects of steam-sterilization and the bacterium on treated samples. Copper loss from in-service treated wood compromises the efficacy of copper-based wood preservatives.

Key words: copper, preservatives, *Bacillus licheniformis* CC0l

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Introduction

The variety of copper-based wood preservatives has increased in recent years because of public concern over potential environmental contamination from the toxic components of inorganic arsenicals. Copper exhibits good biocidal activity (Nicholas and Schultz, 1997), but a major requirement of any formulation of copper-based wood preservatives is based on their efficacy against copper-tolerant fungi. Cole and Clausen (1997) isolated a copper-tolerant bacterium, *Bacillus licheniformis* CC0l from the soil immediately surrounding CCA-treated stakes in a field test plot near Madison, Wisconsin, which exhibited the ability to remove 93 percent of the copper from CCA-treated wood. Do environments with elevated levels of copper select for copper-resistant strains of bacteria or fungi? While much is known about the efficacy and leachability of wood treated with chromated copper arsenate (Cooper 1993; Smith and Shiau 1997), little information is known about the efficacy and leachability of other copper formlations under normal service conditions, Melcher and Peek (1997) conducted a study on the migration behavior in soil of waterborne wood preservatives. They repotted that copper exhibits a high degree of adsorpt ion and low mobility in soil, thereby decreasing it's risk of groundwater contamination. Still, the presence of indigenous copper-resistant microorganisms plays a role in the efficacy of copper-based wood preservatives in service.

It has been reported that exposure to the standard conditions of autoclaving may reduce copper from the cupric (Cu^{+2}) to the cuprous (Cu^{+1}) form, essentially reversing the volubility of the copper component of the preservative (Kamdem and McIntyre, 1997; Kamdem et al. 1998). In copper naphthenate treated southern pine, Kamdem and others report approximately 50% of copper was reduced to cuprous form in samples treated to a retention of 0.31 to 0.51% total copper. To examine the influence of heat on the microbial removal of copper from treated wood, propylene oxide-sterilization was compared to standard autoclave conditions.

Copper-based wood preservatives were selected for this study (Table 1) based on acceptance or pending acceptance of the active ingredient (biocidal component) for use

within the United States. These preservatives have all received Environmental Protection Agency (EPA) approval, Preservatives utilizing copper as their active ingredient represent about 45 percent of all preservation formulations. Copper-based wood preservatives account for approximately 3.46×10^8 cubic feet of treated wood production in the U.S. annually (AWPI, 1997),

The objective of this study was to determine the effect of heat and/or a known copper-tolerant bacterium, Bacillus licheniformis CC0l, on copper in wood treated with six water-borne or oil-borne copper-based preservatives.

Materials and Methods

Preservatives

Table 1. Copper-based wood preservatives.

Copper naphthenate	An oilborne preservative (AWPA, Std P8, 1992) used commercially within the United States.
Copper naphthenate	A waterborne preservative (AWP& Std P8, 1992) used commercially within the United States.
Oxine copper	(Copper-8-Quinolinolate). An oilborne preservative (AWPA, Std P8, 1992) used commercially within the United States.
ACQ-D	A waterborne, amine copper quat system. The American Wood Preservers' Association recently accepted this preservative, The quaternary ammonium is didecyldimethylammonium chloride. The ratio of copper as CuO to Quat is 2:1 (AWPA, Std P5, 1992).
Cu Citrate 2:1	A waterborne, ammoniacal preservative that was being proposed for acceptance by AWPA as this study was initiated. On a weight/weight basis, the molar ratio of copper, as CuO to citrate $(C_6H_4)_7$) is 4:1 On a percentage basis, the CuO:citrate ratio is 2:1 (Anderson et al. 1993).
CCA-C	A waterborne preservative of chromated copper arsenate Type C, having the following composition: hexavalent chromium as CrO_3, 47.5% copper as CuO 18.5%, and arsenic as As_2O_5 34.0% (AWPA, Std P5, 1992)

Preservative Treatement

Southern pine stakes (19 x 19 x 450 mm) were treated at Forest Products Laboratory in Madison, Wisconsin using the full cell process. For each treatment, 1.00% active

ingredient was used to obtained a 0,400 pcf retention. The actual retention (Table 2) was determined by analytical method AWPA Std A l1, 1992,

Copper naphthenate was dissolved in No. 2 diesel oil (Grove 1993), which met AWPA requirements for hydrocarbon solvent Type A (AWPA Std P9, 1992), then diluted with toluene to achieve the desired solution concentration of diesel fuel and active ingredient. All treating solutions with copper naphthenate were mixed with toluene so that the amount of No 2 diesel fuel was only 30% by weight of the total solution. Treating solutions of copper citrate were prepared by diluting the concentrate 2:1 with deionized water. Treating solutions of CCA-type C and ACQ-D were also prepared by diluting concentrates with deionized water.

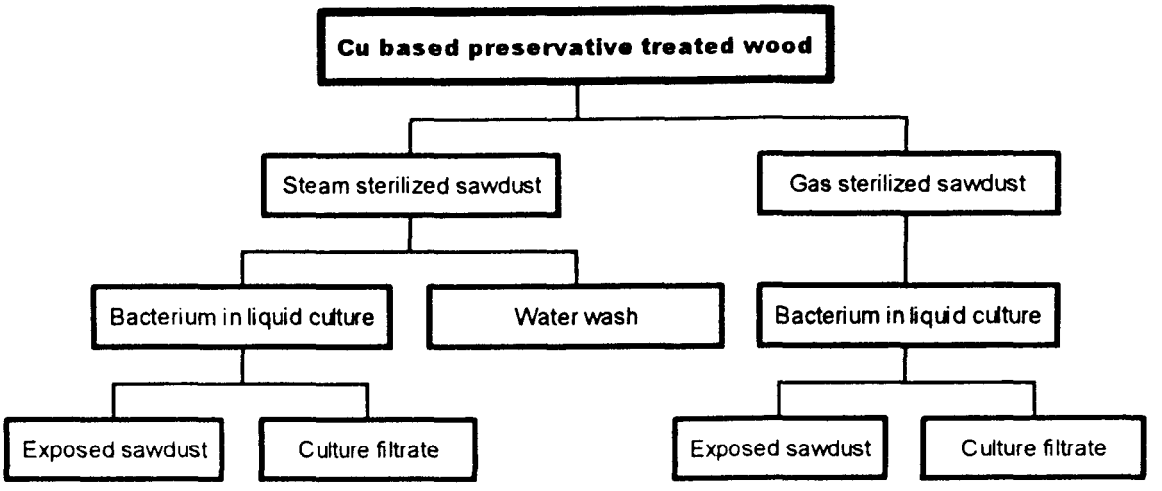
Table 2. Actual retention of the treated southern pine stakes.

Presevatives	*Retention[1] (% total Cu)*
Copper naphthenate in oil	0.340
Copper naphthenate in water	0.496
Oxine copper	0.339
Ammoniacal copper Quat Type D	0.503
Ammoniacal copper citrate	0.468
Chromated copper arsenate Type C	0.400

[1] Retention was determined in accordance with analytical method AWPA Std A11, 1992.

Sterilization and Bacterial treatment

Samples of treated wood were either steam-sterilized or gas-sterilized. For steam-sterilization, one gram samples of treated wood ground to 20-mesh were placed in 300 ml Erlenmeyer flasks containing either 100 ml nutrient broth (0.8%, Difco, Detroit, MI) or 100 ml deionized water and autoclave under standard sterilization conditions of 20 psi, 121 °C for 15 minutes. For gas-sterilization, one gram samples of treated wood were sterilized in closed Erlenmeyer flasks for 18 h by adding 1 ml of propylene oxide (Eastman Organic Chemicals, NY) to a cotton plug in the neck of the flask. One hundred ml sterile nutrient broth were added to each flask following the gas sterilization. Flasks of nutrient broth containing samples of steam- and gas-sterilized wood were inoculated with an 8 h nutrient broth culture of *Bacillus licheniformis* CC0l (Clausen 1997; Clausen and Smith 1998) and incubated for 10 d at 28°C at 150 rpm on a rotating platform. Samples of each type of treated wood sterilized in water were incubated under the same conditions without bacterial inoculum (as diagramed).



Following incubation, the wood was collected with vacuum aspiration through Whatman No. 1 filter paper, dried 24 hr at 60°C, and analyzed for residual copper content by atomic absorption spectroscopy (AA) according to AWPA Al 1-93 (American Wood Preservers' Association 1998). Liquid filtrates of wash water and nutrient growth medium were also analyzed. Samples of wood that were not exposed to heat or bacteria were submitted to determine the base retention of copper, as were samples of gas-sterilized sawdust for comparison with steam-sterilization.

Results and Discussion

Table 3 summarizes the results of steam- or gas-sterilization and bacterial exposure of wood treated with 6 different copper-based wood preservatives. Autoclaving treated wood released as little as 16 percent copper from samples of wood treated with copper naphthenate in water and CCA to as much as 37 percent in samples treated with copper citrate, Propylene oxide-sterilization results demonstrated that gas sterilization followed by exposure to the bacterium, removed as much or more copper than the cumulative effects of steam-sterilization and the bacterium for 4 of the 6 preservative treated wood samples, Control values represent the average of unprocessed and propylene oxide-sterilized controls, since no copper loss was observed in the gas-sterilized samples.

Most of the copper was accounted for in the combined wash filtrate and autoclave wood analysis (Table 3; Figure 1). Exposure of steam-sterilized wood samples to *Bacillus licheniformis* CC0l for 10 d at 28°C released 44 to 82 percent of the remaining residual copper. Again, most of the total copper could be accounted for by the combined analysis of the culture filtrate and wood sample (Figure 1). Likewise, copper totals from gas-sterilized samples approximately equaled the sum of copper from the gas-sterilized samples exposed to bacteria and their filtrates.

Conclusions

Samples of wood treated with six copper-based wood preservatives were either gas- or steam-sterilized and then exposed to the copper-tolerant bacterium, *Bacillus lichenformis* CC0l, Since bacterial cultivation *in vitro* requires steam-sterilization of the culture medium and it has been previously reported that high temperatures increase the reduction of cu^{+2} to Cu^{+1}, microbial studies on treated wood pose two questions 1) Does high temperature reduction of Cu^{+2} to Cu^{+1}, and thus increased solubility, improve the ability of Cu-tolerant bacteria to remove Cu from treated wood? 2) How effective would *Bacillus licheniformis* CC0l beat removing Cu from treated wood in ground contact? Gas-sterilization with propylene oxide demonstrated that the bacterium removed as much or more copper in 4 of 6 gas-sterilized samples than the cumulative effects of steam-sterilization and the bacterium on treated samples. Volubility studies and analysis of the fate of copper following it's release from treated wood by indigenous microbes are needed to predict efficacy of copper-based wood preservatives *in situ.*

References

AWPA. 1992. Standard P5-92. Standards for Waterborne Preservatives. p. 7-10. In: American Wood-Preservers' Association. Book of Standards. American Wood-Preservers' Association, Woodstock, MD. 290 p.

AWPA. 1992. Standard P8-91. Standards for Oil-Borne Preservatives. p. 11-12. In: American Wood-Preservers' Association. Book of Standards. American Wood-Preservers' Association, Woodstock, MD. 290 p.

AWPA. 1992. Standard P9-92. Standard for Solvents and Formulations for Organic Preservative Systems. p. 13-16. In: American Wood-Preservers' Association, Woodstock, MD. 290 p.

AWPA. 1998. Standard A11-93. Standard Method for Analysis of Treated Wood and Treating Solutions by Atomic Absorption Spectroscopy. p. 196-199. In: American Wood-Preservers' Association. Book of Standards. American Wood-Preservers' Association. Granbury, Texas 395 p.

AWPI 1997. The 1996 Wood Preserving Industry Production Statistical Report. American Wood Preservers Institute, Fairfax, VA, September 1997.

Anderson, T.C., R.D. Inwards. R.M. Leach, W.S. McNamara, et al. 1993. Proposal for the Standardization of Ammoniacal copper citrate submitted to AWPA Committee P-4 and The General Preservatives Committee. Osmose Wood Preserving, Inc. Buffalo, NY. 58 p. + Appendices.

Cooper, P.A. (1993) Leaching of CCA: Is it a problem? Disposal of treated wood removed from service: The issues. In: Proceedings, Environmental Considerations in Manufacture,

Use, and Disposal of Preservative-Treated Wood, Carolinas-Chesapeake Section, Forest Products Society, May 13, Richmond, VA.

Clausen, C.A. (1997) Enhanced removal of CCA from treated wood by *Bacillus licheniformis* in continuous culture. *International Research Group on Wood Preservation* IRG/WP/97-50083.

Cole, F.A. and Clausen, C.A. (1997) Bacterial biodegradation of CCA-treated waste wood. In: Proceedings, Forest Products Society Conference on Use of Recycled Wood and Paper in Building Applications, 1996 September 9, Madison, WI.

Clausen, C.A. and Smith, R.L. (1998) Removal of CCA from treated wood by oxalic acid extraction, steam explosion, and bacterial fermentation. *J. Industrial Microbiol. Biotechnol.* 20: 251-257.

Grove, Scott. 1993. Personal communication, OMG, Cleveland, Ohio, September.

Kamdem, D.P. and McIntyre, C.R. (1997) Chemical investigation of 23-year old CDDC treated southern yellow pine. *International Research Group on Wood Preservation* IRG/WP/97-30141.

Kamdem, D.P., Zhang, J., and Freeman, M.H. (1998) The effect of post-steaming on copper naphthenate-treated southern pine. *Wood Fiber and Science*, 30(2):210-217.

Melcher, E. and Peek, R-D (1997) A comparison of the migration behaviour in soil of different waterborne wood preservatives and their leachates. *International Research Group on Wood Preservation* IRG/WP 97-50091.

Nicholas, D.D. and Schultz, T.P. (1997) Comparative performance of several ammoniacal copper preservative systems. *International Research Group on Wood Preservation* IRG/WP 97-30151.

Smith, R.L. and Shiau, R-J. (1997) Steam Processing of Treated Wood for CCA Removal: Identification of opportunities for re-use of the recovered fiber. Southeastern Regional Biomass Energy Program of the Tennessee Valley Authority.

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Table 3. Copper retention in treated southern pine following exposure to steam or gas sterlization and a copper-tolerant bacterium.

Preservative	Retention (pcf)	Treatment sawdust	Treatment liquid	Cu (ppm)	% reduction	Total (ppm)
Cu Nap (oil)	0.340	control		6745		6745
		autoclaved		5000	26	
			H_20 wash	1347		6347
		bacteria		900	82	
			filtrate	4490		5390
		P/bacteria[a]		390	94	
			P/filtrate[b]	6840		7230
Cu Nap (H_20)	0.496	control		8950		8950
		autoclaved		7500	16	
			H_20 wash	1860		9360
		bacteria		2700	64	
			filtrate	6430		9130
		P/bacteria		3490	61	
			P/filtrate	5588		9078
Oxine Cu	0.339	control		1230		1230
		autoclaved		900	27	
			H_20 wash	193		1093
		bacteria		500	44	
			filtrate	476		976
		P/bacteria		710	42	
			P/filtrate	562		1272
ACQ-D	0.503	control		5285		5285
		autoclaved		4000	24	
			H_20 wash	621		4621
		bacteria		800	80	
			filtrate	3840		4640
		P/bacteria		770	85	
			P/filtrate	3769		4539
Cu Citrate	0.468	control		5045		5045
		autoclaved		3200	37	
			H_20 wash	1647		4847
		bacteria		800	75	
			filtrate	3930		4730
		P/bacteria		780	85	
			P/filtrate	5044		5824
CCA	0.400	control		2145		2145
		autoclaved		1800	16	
			H_20 wash	587		2387
		bacteria		400	78	
			filtrate	1907		2307
		P/bacteria		310	86	
			P/filtrate	1496		1806

[a]P/bacteria =proplyene oxide sterilized sample exposed to the bacterium
[b]P/filtrate=filtrate from bacterial culture of propylene oxide sterilized sample

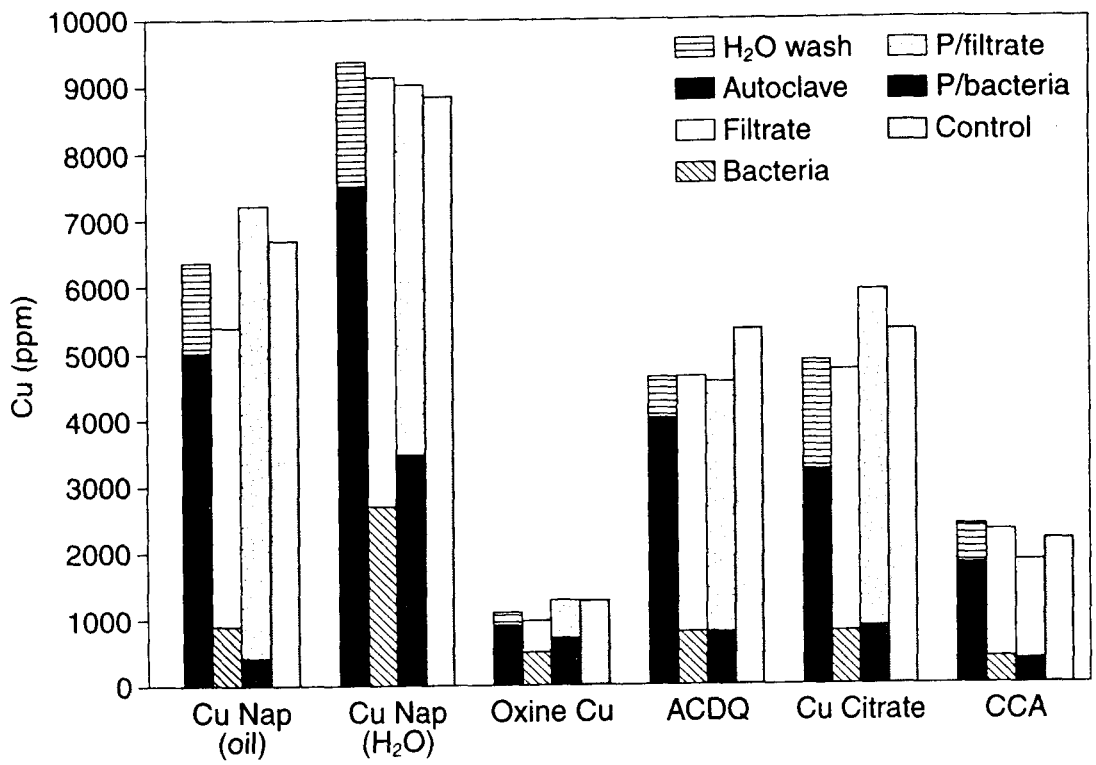

Figure 1. For each preservative, autoclave values represent ppm Cu in wood fiber following steam-sterilization; H_2O wash represents ppm Cu from the liquid that the autoclave wood samples were sterilized in; bacteria values represent ppm Cu in steam-sterilized wood samples following exposure to the bacterium; filtrate values represent ppm Cu in the bacterial nutrient growth medium. Control values indicate the average total ppm copper in unprocessed samples; P/bacteria represents ppm Cu in wood fiber of gas-sterilized samples following exposure to the bacterium; P/filtrate values represent ppm Cu in the bacterial nutrient broth medium of gas-sterilized samples. Note: autoclave plus H_2O wash should theoretically equal the control value. Likewise, bacteria plus filtrate and P/bacteria plus P/filtrate should theoretically equal the control value.